1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on August 28, 2009 has been entered.

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

3. Claims 14, 17-21, 23, 26-28 and 43-53 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chan or Yu (Biochemistry, 2001, hereinafter called Yu-B) in view of Heegaard or Yu (Analytical Chemistry, 2001, hereinafter called Yu-A).

In the paper Chan teaches the construction and characterization of a heterodimeric iron protein complex. The heterodimeric protein complex is made up of two protein subuints that have MgATP binding sites. Separation of the heterodimeric Fe protein from a mixed population with homodimeric Fe proteins contained in crude extracts was accomplished by construction of a seven His tag on one subunit and a differential immobilized-metal-affinity chromatography technique. Page 7223 has a section on the purification in which it teaches that His-tagged Fe proteins were further purified by a Zn-affinity chromatography system (24), with step gradient elution with buffer containing either 75 or 250 mM imidazole. the first two paragraphs of page 7224 teach in part that assuming equal expression from both copies of the nifH gene and random assembly of the dimeric Fe protein, a mixed population of homodimeric and heterodimeric Fe

proteins should accumulate. These include a wild-type homodimeric [Asp^{39}/Asp^{39}] Fe protein (this protein is made up of two second protein subunits), an altered homodimeric [Asn^{39}/Asn^{39}] Fe protein, and a heterodimeric [Asp^{39}/Asn^{39}] Fe protein (this protein is made up of first and second protein subunits). The homodimeric [Asp^{39}/Asp^{39}] Fe protein carries a His tag on the N-terminus of both subunits, whereas the heterodimeric [Asp^{39}/Asn^{39}] Fe protein contains a His tag on only one of the subunits. The altered homodimeric [Asn^{39}/Asn^{39}] Fe protein does not contain a His tag on either subunit. The presence or absence of His tags located on the various Fe protein types present in a mixed population was used to specifically purify the heterodimeric [Asp^{39}/Asn^{39}] Fe protein. Fe proteins were first collectively purified from crude extracts of strain DJ1274 using traditional ion-exchange and gel-filtration chromatography (17). The purified proteins were then applied to a Zn-affinity column. A first fraction of the loaded Fe protein eluted from the column upon washing with buffer containing no imidazole. This protein was shown to be the altered homodimeric [Asn^{39}/Asn^{39}] Fe protein for which neither subunit carries a His tag. A second fraction of Fe protein was eluted from the column when washed with a buffer containing 75 mM imidazole. This protein was shown to be the heterodimeric [Asp^{39}/Asn^{39}] Fe protein that carries a His tag on only one of the subunits (elution of the first protein subunit). A third fraction of Fe protein was subsequently eluted from the column by washing with buffer containing 250 mM imidazole. This protein was shown to be the wild-type homodimeric [Asp^{39}/Asp^{39}] Fe protein that contains a His tag on both subunits (elution of the second protein subunit). A diploid strain (DJ1298) that produces a mixed population of His-tagged Fe proteins that do not have any amino acid substitutions was also constructed. By using strain DJ1298 and the purification strategy described above it was possible to isolate a homodimeric [Asp^{39}/Asp^{39}] Fe protein for which only one of the subunits carries a His tag which was used for control experiments. Chan does not teach that the extraction channel is an open channel having an extraction surface on the inner surface of the channel.

In the paper Yu-B teaches the construction and characterization of a homotetrameric enzyme (protein complex). Page 15583 discusses the expression and purification of the homomutant and heteromutant protein complexes. the complexes were made to include a with the addition of a six-histidine tag. In the case of the homomutant complex a crude extract was applied to a Ni-affinity column (His-Bind^{R} resin, Novagen) precquilibrated with Buffer A (20

mM Tris-HCl, pH 8, 0.5 M NaCl). The column was washed with 30 mL of 5 mM imidazole in Buffer A and 12 mL each of 30 and 60 mM imidazole in Buffer A. The protein was eluted with 15 mL of 100 mM imidazole in Buffer A. The heteromutant proteins were also expressed and applied to the Ni-affinity column as described above for the homomutant proteins. The column was washed with 30 mL of Buffer A, 30 mL of Buffer A with 5 mM imidazole, and 12 mL each of Buffer A with the following imidazole concentrations: 10, 20, 30, 60, 100, and 200 mM. The fractions eluted with 30 and 60 mM imidazole were pooled and dialyzed overnight at 4 °C against 4 L of dialysis buffer. All proteins were approximately 95% pure. The paragraph bridging pages 15583-15584 teaches that to facilitate purification of wild-type and mutant ASL proteins, the proteins were expressed with C-terminal 6-His tags. 6-His-tagged wild-type ASL and Q286R and D87G ASL mutants (wtASL-his, Q286R-his, and D87G-his, respectively) were expressed in E. coli and yielded approximately 10−20 mg of 95% pure protein per liter of cultured cells (Figure 1a). Plasmids were constructed to coexpress various pairs of mutants within the same E. coli cell. On each plasmid, only one of the mutant ASL genes encoded a C-terminal 6-His tag. The objective of this approach was to produce heterotetramers in vivo, which could subsequently be purified by Ni-affinity chromatography. There are five different heterotetramers that can be formed from the combination of two different monomers (mutants 1 and 2). These tetramers would contain mutant 1 and mutant 2 polypeptides in the following ratios: 0:4, 1:3, 2:2, 3:1, 4:0. Since only one of the two mutants has a 6-histidine tag, the tetramers produced are expected to have varying affinities for a Ni column based on the number of histidine-tagged subunits present. the following paragraph on page 15584 in combination with figure 1c shows that this is the case. Yu-B does not teach that the extraction channel is an open channel having an extraction surface on the inner surface of the channel.

In the paper Heegaard discusses affinity capillary electrophoresis. Relevant to the instant claims is section 4.1 discussion on-line solid-phase extraction or preconcentration chambers. Figures 13A and 14 show the simplest enrichment chamber built to date. It is described in the paragraph bridging pages 43-47 and is made of a portion of a capillary containing an immobilized selector that is used as an affinity ligand to capture a specific analyte. One form of the selector is taught as an antibody (page 43). Because the antibody recognizes a specific portion of the antigen (or hapten) molecule, it is highly specific. Therefore, the results

demonstrated a substantial increase in concentration of analytes when using this approach. Important advantages of this on-line concentrator design are the following: (a) the binding of the antibody to the antigen is uniform, providing identical association and dissociation kinetics; (b) the design has no beads or frits, diminishing the chance of blocking or clogging the enrichment chamber; and (c) there is a more uniform flow-rate, allowing for more reproducible results. Page 45 and 47 teach two other types of concentrators utilizing surface-bound octadodecylsilane for the separation of herbicides and surface-bound metal chelating groups for the separation of proteins that exhibit an affinity for the chelating metal.

In the paper Yu-A teaches the preparation of monolithic porous polymers within the channels of a microfluidic device for on-chip solid-phase extraction and preconcentration. The preparation of the monolithic material (page 5090-5091)with hydrophobic and ionizable surface chemistries is easily achieved by copolymerization of butyl methacrylate with ethylene dimethacrylate, or 2-hydroxyethyl methacrylate and [2-(methacryloyloxy)ethyl] trimethylammonium chloride with ethylene dimethacrylate, respectively. The porous properties, and consequently the flow resistance, of the monolithic device are controlled by the use of a mixture of hexane and methanol as a porogenic mixture. This mixture was designed to meet the specific requirements for pore formation within macroporous monoliths useful in the microfluidic formats. The low flow resistance enables high flow rates of up to 10 μL/min, which corresponds to a linear flow velocity of 50 mm/s and far exceeds the flow velocities typical of the common analytical microchips. The function of the monolithic concentration device was demonstrated with the enrichment of a hydrophobic tetrapeptide and also of green fluorescent protein for which an increase in concentration by a factor as high as 103 was achieved.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use the open channel/capillary concentrators of Heegaard or Yu-A in the methods of Chan or Yu-B because of the flow and other advantages taught by Heegaard and Yu-A. Relative to the nonlinear or coiled shape of the channel, the Court in In re Dailey, 149 USPQ 47 (CCPA 1966) held that the configuration of the claimed disposable plastic nursing container was a matter of choice which a person of ordinary skill in the art would have found obvious absent persuasive evidence that the particular configuration of the claimed container was significant. Relative to the plurality of channels, the Court in In re Harza, 124 USPQ 378 (CCPA 1960) held

that mere duplication of parts has no patentable significance unless a new and unexpected result is produced.

4. Claims 15-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chan or Yu-B in view of Heegaard or Yu-A as applied to claim 14 above, and further in view of Boettger (US 4,221,568) or Gobom. Neither Chan nor Yu-B expressly teach purging the extraction channel with a gas prior to passing a desorption solution through the channel wherein the extraction surface remains substantially solvated after the purging step.

In the patent Boettger teaches an apparatus that can process numerous samples that must be chemically analyzed by the application of fluids such as liquid reagents, solvents and purge gases, as well as the application of dumps for receiving the applied fluid after they pass across the sample, in a manner that permits numerous samples to be processed in a relatively short time and with minimal manpower. The processor includes a rotor which can hold numerous cartridges (tubes, figure 3) containing inert or adsorbent material for holding samples, and a pair of stators on opposite sides of the rotor. The stators form stations spaced along the path of the cartridges which lie in the rotor, and each station can include an aperture in one stator through which a fluid can be applied to a cartridge resting at that station, and an aperture in the other stator which can receive the fluid which has passed through the cartridge. Columns 5-6 teach one example of a protocol involving purging of the sample cartridges during the process.

Gobom teaches a capillary column (Figure 1) comprised of fused silica (p. 107). Gobom teaches that the advantage of using the capillary column is that it is simple, fast and utilizes only low-cost disposables (abstract). In addition, it allows efficient sample concentration and the elution process yields very small sample spots. Gobom also teaches using a method of extracting proteins wherein the extraction channel is purged with a gas before adding the desorption solution (page 107, right column). This step this step is imperative for smooth and continuous liquid flow in the next step (abstract, page 107, right column).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Chan or Yu-B by purging the channel with gas before adding the desorption solution in order to gain the predictable results of a smooth and continuous liquid flow during the purification of the protein as taught by Gobom or to increase the efficiency of the process as taught by Boettger.

5. Claims 22 and 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chan or Yu-B in view of Hecgaard or Yu-A as applied to claim 19-20 above, and further in view of Bornhorst. Chan or Yu-B do not teach the inclusion in at least one of the desorption solutions of an agent that will affect protein-protein interactions.

In the paper Bornhorst teaches various aspects of protein purification using polyhistidine affinity tags. The expression and subsequent purification of recombinant proteins are widely employed in biochemical studies. A powerful purification method involves the use of peptide affinity tags, which are fused to the protein of interest and used to expedite protein purification via affinity chromatography. A widely employed method utilizes immobilized metal-affinity chromatography (IMAC) to purify recombinant proteins containing a short affinity tag consisting of polyhistidine residues. Figure 1 on page 248 shows two common affinity matrices used in the purification. The paragraph bridging pages 248-249 teaches that purification using the polyhistidine tag can be performed under either native or denaturing conditions by IMAC. The use of mild buffer conditions and imidazole as the eluant often yields biologically active purification products. Proteins that remain soluble in the cytoplasm, or that are secreted, usually can be purified using these native conditions. However, purification under native conditions may be hindered if the target protein is insoluble, aggregates in inclusion bodies, or possesses a tertiary structure that occludes the polyhistidine affinity tag. In such cases, proteins can be purified by the use of denaturing conditions such as 6 M guanidinium hydrochloride or 8 M urea during the purification process. Interaction of the resin with the polyhistidine tag does not require a specific conformation of the peptide tag, which makes effective purification with the use of denaturing conditions possible. Purification under denaturing conditions can also depress the activity of phosphatases and proteolytic enzymes.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate an agent such as urea or guanidine hydrochloride into the desorption solutions of Chan or Yu-B because as taught by Bornhorst there are situations in which protein purification requires the protein-protein interactions to be removed in order to facilitate the purification using a polyhistidine affinity tag.

6. Claims 41-42 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

7. The following is a statement of reasons for the indication of allowable subject matter: the art of record fails to teach or fairly suggest the combination of method steps found in claims 41-42.

8. Applicant's arguments with respect to the claims have been considered but are moot in view of the new ground(s) of rejection.

9. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to protein purification using resin-based extraction/affinity agents.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571)272-1265. The examiner can normally be reached on Monday-Thursday and Alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Vickie Kim can be reached on (571) 272-0579. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Arlen Soderquist/
Primary Examiner, Art Unit 1797